

Anesthetic Influence on Arteriolar Diameters and Tissue Oxygen Tension in Hemorrhaged Rats

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Arteriolar diameters and tissue oxygen tensions were measured in the cremaster muscles of 68 hemorrhaged rats which were anesthetized with either intramuscular ketamine, 125 mg/kg, plus 30 mg/kg supplements as needed, or enflurane, 2.2% inspired. Animals breathed room air, or room air plus enflurane, throughout the experiments. Arterioles in the cremaster muscle were identified according to successive orders of branching, and the internal diameters of first-, third-, and fourth-order vessels were measured at 30-s intervals. Cremaster muscle oxygen tension was measured polarographically with platinum-iridium microelectrodes. Mean arterial pressure was controlled at 30-35 mmHg during 30 min of hemorrhage, and maximum shed blood volumes were similar (2.6 ml/100 g) in both groups. Principal responses to hemorrhage in rats receiving enflurane were 1) constriction in first-, third-, and fourth-order arterioles, and 2) tissue hypoxia. In hemorrhaged rats receiving ketamine, the constrictor response to hemorrhage either was diminished (first- and third-order arterioles) or abolished (fourth-order arterioles), and tissue hypoxia did not occur. The authors conclude that ketamine, as compared with enflurane, diminishes or prevents arteriolar constriction and tissue hypoxia in the cremaster muscle of hemorrhaged rats. (Key words: Anesthetics, intravenous: ketamine. Anesthetics, volatile: enflurane. Arteries: arterioles. Hemorrhage. Microcirculation: muscle. Oxygen: tension, muscle. Shock: hemorrhagic.)

RESULTS OF PREVIOUS EXPERIMENTS indicate that anesthetics influence the survival of rats subjected to hemorrhage.¹ Peripheral circulatory failure and consequent tissue hypoxia are prominent features of hemorrhagic shock, and drugs which alter peripheral circulatory function have a marked influence on survival following hemorrhage.^{2,3} We reasoned, therefore, that anesthetics might also influence the peripheral vascular responses to hemorrhage. There is indirect evidence to support this contention, since anesthetics alter the quantity of excess lactate in the arterial blood following hemorrhage, suggesting that the degree of tissue ischemia accompanying hypovolemia is altered by the anesthetics as well.^{4,5} The

present study was designed to test the hypothesis that anesthetics alter microvascular control and tissue oxygenation in the striated musculature of hemorrhaged rats.

Methods

Sixty-eight young male Sprague Dawley rats (body weights 112 ± 3 g) were divided into two groups based on anesthetic exposure. Rats were anesthetized with either intramuscular ketamine, 125 mg/kg initially plus 30 mg/kg supplements as required, or with enflurane, 2.2 volumes per cent (inspired) delivered from a previously calibrated vaporizer.§ The anesthetic doses were identical to those used in previous hemorrhage studies in this species.^{1,4,5} Animals breathed room air (or air plus enflurane) spontaneously throughout the experiments.

Within each anesthetic group, animals were subdivided into those used for microvascular diameter measurements and those in which tissue oxygen tension was measured. In the microvascular experiments, the diameter of either a first-, third-, or fourth-order arteriole in the cremaster muscle was measured by closed-circuit television microscopy. The video display included movable cursors which could be positioned over the vessel walls and the distance between the cursors was presented on a digital display.¶ System accuracy was $\pm 0.5\%$. Details of the method have been reported previously.⁶ Since, the responses of second-order arterioles were reported to be similar qualitatively to those of first-order arterioles,⁷ the second-order vessels were not measured in our experiments. Cremaster muscle oxygen tension was measured polarographically using conical, polystyrol-coated, platinum-in-glass oxygen microelectrodes (tip diameters approximately $1 \mu\text{m}$) constructed according to the method of Silver.⁸ The oxygen cathodes were polarized at 0.7 volts relative to a Ag-AgCl reference electrode, and the resultant current was measured with a sensitive picoammeter.** The electrodes were positioned at locations which were remote from major arterioles or venules in order to obtain values which represented tissue, rather than perivascular, oxygen tension. The output of these

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§ Drager Vapor, North American Draper Corp., Telford, Pennsylvania 18969.

¶ Circon Corp., Santa Barbara, California 93111.

** Chemical Microsensor Model 1201, Transidyne General Corp., Ann Arbor, Michigan 48103.

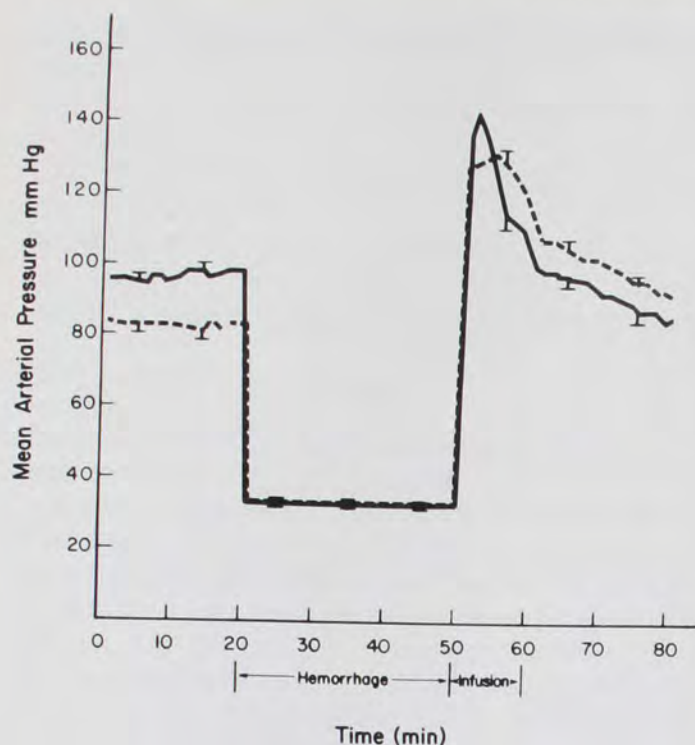


FIG. 1. Mean arterial pressure before, during, and after hemorrhage in rats anesthetized with either ketamine ($N = 38$; solid line) or enflurane ($N = 30$; dashed line). Arterial pressure was controlled at 34 mmHg during hemorrhage by a servo-syringe system. The increase in arterial pressure during infusion of the shed blood (50–60 min) resulted from the arterial transfusion. Before hemorrhage, arterial pressure was significantly greater ($P < 0.001$) in rats anesthetized with ketamine as compared with those breathing enflurane.

microelectrodes (approximately 10^{-9} to 10^{-10} A) is linear and directly proportional to the oxygen tension. The system can resolve changes in oxygen tension of one to three per cent of the measured value (0.1 to 0.4 mmHg in these studies). During the oxygen tension experiments, the cremaster muscle was suffused with a physiologic salt solution,†† which was equilibrated with nitrogen to prevent oxygen from diffusing into the tissue from the atmosphere, a technique which has been used by others when measuring oxygen tension in thin tissues.⁹

The experimental preparation consisted of the following sequence: anesthetic induction, carotid artery cannulation and surgical preparation of the cremaster muscle (approximately 60 min), a 60- to 90-min period to allow recovery of the cremasteric microvasculature following surgical manipulation and to permit anesthetic equilibration, followed by the experimental protocol. The experimental protocol consisted of a 20-min control period, 30 min of hemorrhage during which mean arterial pressure was controlled at 30–35 mmHg by a servo-controlled syringe system, a 10-min infusion period

during which the shed blood was returned to the animal through the arterial cannula, and a final 20-min observation period. Mean arterial pressure, shed blood volume, and tissue oxygen tension were measured continuously and recorded at one-minute intervals. Arteriolar diameters were measured every 30 s. Body temperature was controlled at 37°C, and cremaster muscle temperature was maintained at 34.5°C (the physiologic temperature for this tissue).⁷

Responses during hemorrhage were compared to their respective control values by Student's *t* test for paired data, and responses between anesthetics (ketamine *vs.* enflurane) were compared by Student's *t* test for unpaired data. A probability of 0.05 was accepted as the minimal level of statistical significance. Since the control arteriolar diameters differed between anesthetic groups, these values were normalized to per cent of control by dividing each value by the average diameter during the 20-min control period, and multiplying the dividend by 100.

Results

All animals survived the period of hypovolemia. Figure 1 depicts the mean arterial pressures before, during, and after hemorrhage. Before hemorrhage, the mean arterial pressure was greater in animals anesthetized with ketamine as compared with those receiving enflurane (96 ± 2 mmHg *vs.* 83 ± 2 mmHg; $P < 0.001$). Arterial pres-

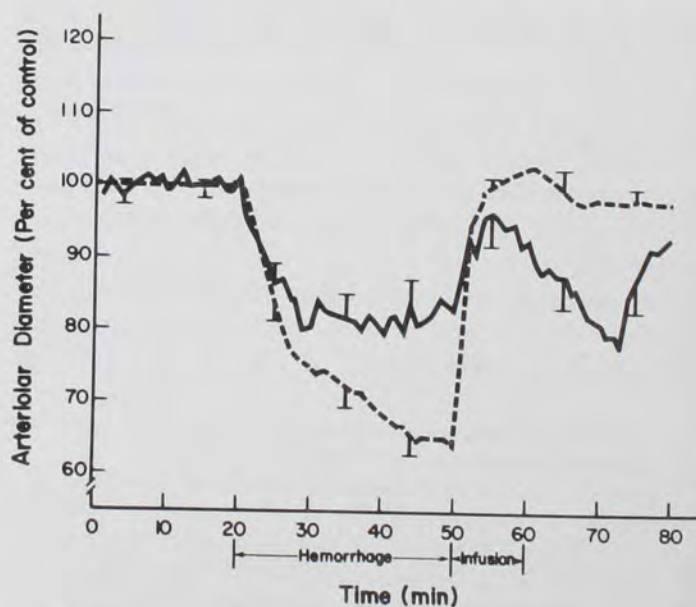


FIG. 2. Diameters of first-order arterioles before, during, and after hemorrhage in rats anesthetized with either ketamine ($N = 12$; solid line) or enflurane ($N = 7$; dashed line). Before hemorrhage, first-order arterioles were larger in animals breathing enflurane as compared with those receiving ketamine ($125 \pm 5 \mu\text{m}$ *vs.* $95 \pm 3 \mu\text{m}$; $P < 0.001$). Constriction ($P < 0.05$) occurred in both anesthetic groups during hemorrhage, but the response was significantly less in those receiving ketamine.

†† Polyonic R 148, Cutter Laboratories, Inc., Berkeley, California 94710.

pressure was controlled during hemorrhage and the values were essentially identical in animals receiving either ketamine or enflurane (34 ± 1 mmHg vs. 33 ± 1 mmHg, respectively). After hemorrhage, the arterial pressures were not significantly different between animals receiving ketamine and those breathing enflurane.

The shed blood volumes were not significantly different between the anesthetic groups. Maximum shed blood volumes were 2.5 ± 0.1 ml/100 g body weight in rats anesthetized with ketamine, and 2.6 ± 0.1 ml/100 g in those receiving enflurane.

Figure 2 illustrates the responses of first-order arterioles to hemorrhage during either enflurane ($N = 7$) or ketamine ($N = 12$) anesthesia. Prior to hemorrhage, first-order arterioles were larger in animals breathing enflurane as compared with those receiving ketamine (125 ± 5 μ m vs. 95 ± 3 μ m, respectively; $P < 0.001$). Significant arteriolar constriction ($P < 0.05$) was evident within two minutes of the onset of hemorrhage and persisted throughout hemorrhage in all animals. The constrictor response was greater in animals receiving enflurane as compared with those receiving ketamine ($P < 0.05$; 22 through 50 min inclusive). However, the minimum caliber of first-order arterioles was similar in both groups during hemorrhage (80 ± 5 μ m vs. 76 ± 2 μ m; enflurane and ketamine, respectively).

Figure 3 depicts the responses of third-order arterioles

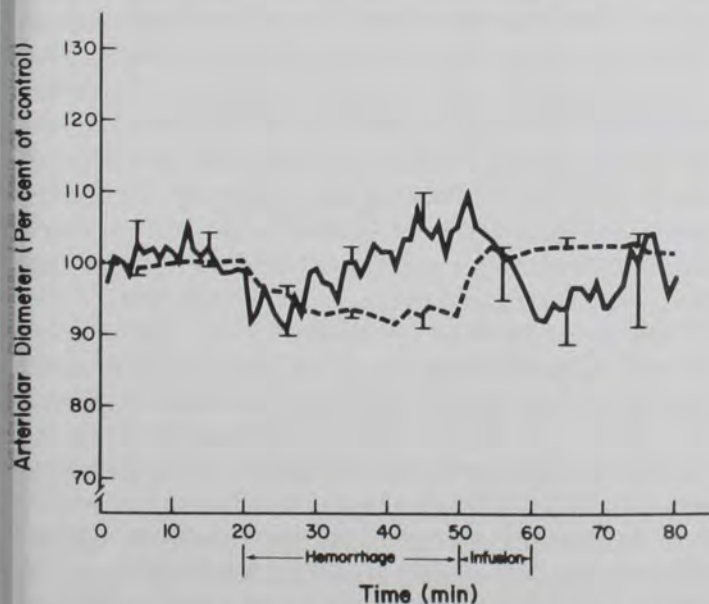


FIG. 3. Diameters of third-order arterioles before, during, and after hemorrhage in rats anesthetized with either ketamine ($N = 6$; solid line) or enflurane ($N = 6$; dashed line). Before hemorrhage, third-order arteriolar caliber was greater in rats breathing enflurane as compared with those receiving ketamine (54 ± 1 μ m vs. 48 ± 3 μ m; $P < 0.05$). Significant constriction persisted throughout hemorrhage in animals receiving enflurane, but this was only transient in those anesthetized with ketamine. Responses were significantly different ($P < 0.05$) between anesthetic groups during the last 13 min of hemorrhage.

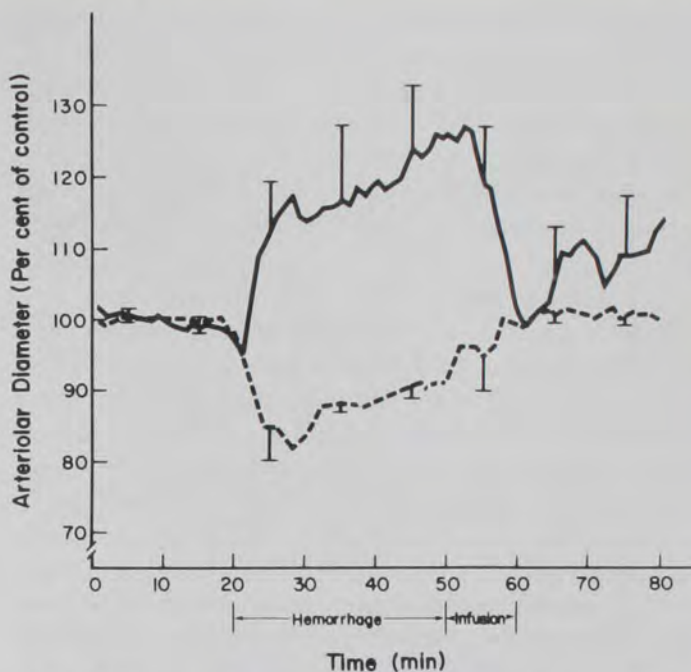


FIG. 4. Diameters of fourth-order arterioles before, during, and after hemorrhage in rats anesthetized with either ketamine ($N = 9$; solid line) or enflurane ($N = 7$; dashed line). Before hemorrhage, fourth-order arteriolar diameters were significantly greater in animals breathing enflurane as compared with those receiving ketamine (24 ± 1 μ m vs. 21 ± 1 μ m; $P < 0.05$). Arterioles constricted significantly ($P < 0.05$) throughout hemorrhage in animals breathing enflurane, but these vessels dilated ($P < 0.05$) during the last 11 min of hemorrhage in those receiving ketamine. Fourth-order arteriolar responses were significantly different ($P < 0.05$) between anesthetic groups throughout hemorrhage.

to hemorrhage during either ketamine ($N = 6$) or enflurane ($N = 6$) anesthesia. Prior to hemorrhage, the arteriolar diameters were significantly greater in rats breathing enflurane as compared with those receiving ketamine (54 ± 1 μ m vs. 48 ± 3 μ m, respectively; $P < 0.05$). Hemorrhage in animals anesthetized with ketamine elicited brief initial third-order arteriolar constriction ($P < 0.05$; 21 through 26 min inclusive) followed by a trend toward, but statistically insignificant, dilation later in hemorrhage. In animals breathing enflurane, the third-order arterioles constricted significantly throughout hemorrhage ($P < 0.05$; 21 through 50 min inclusive). Responses were significantly different between the anesthetic groups during the later part of the hypovolemic period ($P < 0.05$; 38 through 50 min).

Figure 4 portrays the responses of fourth-order arterioles to hemorrhage during enflurane ($N = 7$) or ketamine ($N = 9$) anesthesia. Prior to hemorrhage, fourth-order arteriolar diameters were greater in animals breathing enflurane as compared with those receiving ketamine (24 ± 1 μ m vs. 21 ± 1 μ m, respectively; $P < 0.05$). In animals breathing enflurane, the fourth-order arterioles constricted throughout hemorrhage ($P < 0.05$; 21 through 50 min) although the magnitude of

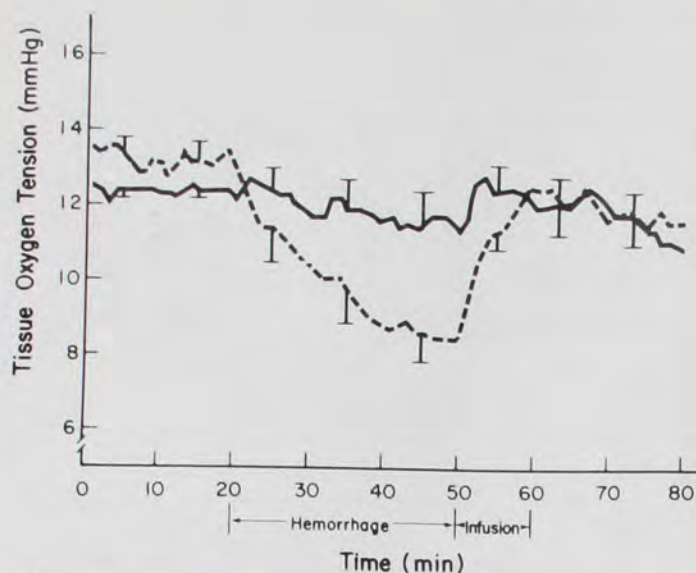


FIG. 5. Cremaster muscle oxygen tension before, during, and after hemorrhage in rats anesthetized with ketamine ($N = 11$; solid line) or enflurane ($N = 10$; dashed line). Prehemorrhage values were similar during ketamine or enflurane anesthesia (12.4 ± 0.2 mmHg and 13.3 ± 0.4 mmHg, respectively). Tissue PO_2 decreased significantly ($P < 0.05$) throughout hemorrhage in animals breathing enflurane, but it was unchanged in those receiving ketamine. Responses were significantly different ($P < 0.05$) between anesthetic groups during the last 15 min of hypovolemia.

this constriction appeared to wane during the later part of the hypovolemic period. During ketamine anesthesia, hypovolemia resulted in progressive fourth-order arteriolar dilation which was statistically significant during the last eleven minutes of hemorrhage ($P < 0.05$; 40 through 50 min). Fourth-order arteriolar responses were significantly different between anesthetic groups essentially throughout hemorrhage ($P < 0.05$; 23 through 50 min).

Figure 5 depicts the cremaster muscle oxygen tensions before, during, and after hemorrhage in animals anesthetized with either enflurane ($N = 10$) or ketamine ($N = 11$). Before hemorrhage, muscle oxygen tension values were similar during either enflurane or ketamine anesthesia (13.3 ± 0.4 mmHg and 12.4 ± 0.2 mmHg, respectively). In animals breathing enflurane, muscle oxygen tension decreased significantly essentially throughout hemorrhage ($P < 0.05$; 23 through 50 min). Cremaster muscle oxygen tension was unchanged during hemorrhage in rats anesthetized with ketamine. Muscle oxygen tensions were significantly different between the anesthetic groups during the last 15 min of hypovolemia ($P < 0.05$ 36 through 50 min).

Discussion

The results indicate that ketamine, as compared with enflurane, reduces the magnitude of the precapillary va-

soconstrictor response and prevents tissue hypoxia in the cremaster muscles of rats subjected to moderate hemorrhage. The differences cannot be explained by differences in animal weights (and therefore ages) or gender, nor by differences in arterial pressures or shed blood volumes during hemorrhage. Neither ketamine (unpublished observations) nor enflurane¹⁰ alters the response of oxygen microelectrodes. The results appear to represent anesthetic-induced alterations in the peripheral circulatory responses to hemorrhage.

An alternate explanation of our results would be that enflurane enhanced the arteriolar constrictor response, and not that ketamine reduced it. This point is difficult to establish in the absence of hemorrhage studies in unanesthetized rats. However, the constrictor responses to hemorrhage during enflurane which we report here are similar, both qualitatively and quantitatively, to those which we reported previously in hemorrhaged rats receiving halothane.^{11,12} Further, the microvascular responses during enflurane are similar to those reported by Hutchins, Goldstone, and Wells, who observed the cremaster microvascular of hemorrhaged rats which were anesthetized with a combination of chloralose and urethane.⁷ Thus, ketamine appears to differ from both inhalation anesthetics and other intravenous anesthetics in this regard.

Ketamine, as compared with enflurane, reduced or abolished the arteriolar constrictor response to hemorrhage. This effect was evident at all levels in the microvasculature but it was most dramatic in the smaller arterioles. In first-order arterioles, ketamine reduced the magnitude of arteriolar constriction. In third-order arterioles, ketamine prevented vasoconstriction during all except the first six minutes of hemorrhage. In fourth-order arterioles, ketamine anesthesia resulted in arteriolar dilation during hemorrhage while precapillary constriction occurred in animals receiving enflurane.

The mechanisms which account for the arteriolar responses to hemorrhage, or the effects of anesthetics on these responses, have not been clearly defined. In general, the smaller arterioles are more responsive to vasoactive substances than are the larger ones,¹³ while the larger vessels tend to be more responsive to neural control.¹⁴ The sympathetic activation which accompanies hemorrhage should activate both neural and humoral responses, and the associated decrease in arterial pressure could result in passive precapillary constriction also. Since ketamine eliminated the constrictor responses to hemorrhage in third- and fourth-order arterioles, it is possible that the constriction which we observed in the first-order arteries of animals receiving ketamine anesthesia was a passive phenomenon. Pressure-diameter studies in isolated perfused arterioles are consistent with this possi-

bility. When perfusion pressure in isolated 100- μ m arterioles was decreased from approximately 100 to 40 mmHg, the decrease in arteriolar diameter was similar to that which we observed during hypotension in animals receiving ketamine.^{††} At present, we are unaware of other experimental data which would either confirm or refute this hypothesis.

Cremaster muscle oxygen tensions were similar in normovolemic animals breathing enflurane and in those receiving ketamine, indicating that systematic differences in tissue oxygen tension did not account for the differences which occurred during hemorrhage. The control tissue oxygen tension values reported here are almost identical to those reported by others using similar experimental preparations.⁹

Hemorrhage during enflurane anesthesia resulted in muscle tissue hypoxia, but this did not occur in rats receiving ketamine. The results imply that ketamine and enflurane have different effects on the ratio of oxygen supply to oxygen demand during hemorrhage. Oxygen supply could be influenced by a number of factors, including arterial hypoxemia, alterations in hemoglobin affinity for oxygen, and regional distributions of blood flow. While these factors were not measured in our study, results from previous studies demonstrated that arterial hypoxemia did not occur even after 60 min of hemorrhage in rats anesthetized with either ketamine or enflurane.^{4,5} Therefore, arterial hypoxemia alone cannot explain the differences in tissue oxygen tension reported here. While we did not establish a direct cause-and-effect relationship between arteriolar responses and tissue oxygen in these studies, it appears likely that the tissue hypoxia seen during hemorrhage in animals receiving enflurane was a direct result of arteriolar constriction, and that ketamine prevented the development of tissue hypoxia by blocking precapillary vasoconstriction during hemorrhage.

Ketamine, as compared with halothane¹ or enflurane (unpublished observations), is associated with reduced mortality following hemorrhage in rats. It is likely that the microvascular responses observed in these studies are at least partially responsible for the improved survival following hemorrhage in rats receiving ketamine as compared with those receiving enflurane. Previous investigations suggested an inverse relationship between the intensity of peripheral vasoconstriction and the probability of survival following hemorrhage (*i.e.*, more intense vasoconstriction resulted in greater mortality).^{2,3} Thus, norepinephrine infusion during hemorrhage in dogs resulted in increased arterial pressure but also increased

mortality,² presumably due to decreased nutrient blood flow to tissues resulting from norepinephrine-induced arteriolar constriction. A similar mechanism apparently explains the increased mortality following hemorrhage during cyclopropane as compared with halothane or iso-flurane anesthesia.¹⁵ The results of the present study and of our previous survival studies are consistent with this hypothesis.

A lesser, but nevertheless interesting, finding in this study was the consistent difference in arteriolar diameters between normovolemic animals receiving ketamine and those breathing enflurane. Since anesthetics have been shown to alter microvascular diameters in other tissues,^{16,17} individual arterioles were selected for observation based on the level of branching, and not on a predetermined diameter basis. At each microvascular level (first-, third-, and fourth-order), the arterioles were larger in animals anesthetized with enflurane as compared with those receiving ketamine. This systematic difference may represent differing microvascular effects of these drugs in normovolemic animals. We are unaware of other experiments which have directly compared the muscle microvascular effects of enflurane versus ketamine.

We conclude the following from these studies: 1) hemorrhage during enflurane anesthesia resulted in cremaster muscle arteriolar constriction and tissue hypoxia, but 2) the vasoconstrictor response was diminished (first-, and third-order arterioles) or abolished (fourth-order arterioles) and tissue hypoxia did not occur in the cremaster muscle of hemorrhaged rats receiving ketamine. Several caveats should accompany these conclusions: 1) the data are for one tissue and one anesthetic dose only, 2) the response in awake animals is unknown, so it is not known whether ketamine preserves or alters the "normal" response, and 3) the results apply to moderate hemorrhage. Obviously, if either the degree or duration of hemorrhage were increased, one would expect to reach a point where the apparent protective effect of ketamine would be overwhelmed by the magnitude of hypovolemia and tissue hypoxia would result.

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